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CONTENTS

		PAGE
UHLENHUTH, EDWARD	*Observations on the Distribution and Habits of the Blind Texan Cave Salamander, Typhlomolge Rathbuni*	73
NEWMAN, H. H.	*On the Development of the Spontaneously Parthenogenetic Eggs of Asterina (Patiria) Miniata*	105
NEWMAN, H. H.	*On the Occurrence of Paired Madreporic Pores and Pore-Canals in the Advanced Bipennaria Larvæ of Asterina (Patiria) Miniata together with a Discussion of the Significance of Similar Structures in Other Echinoderm Larvæ*	118

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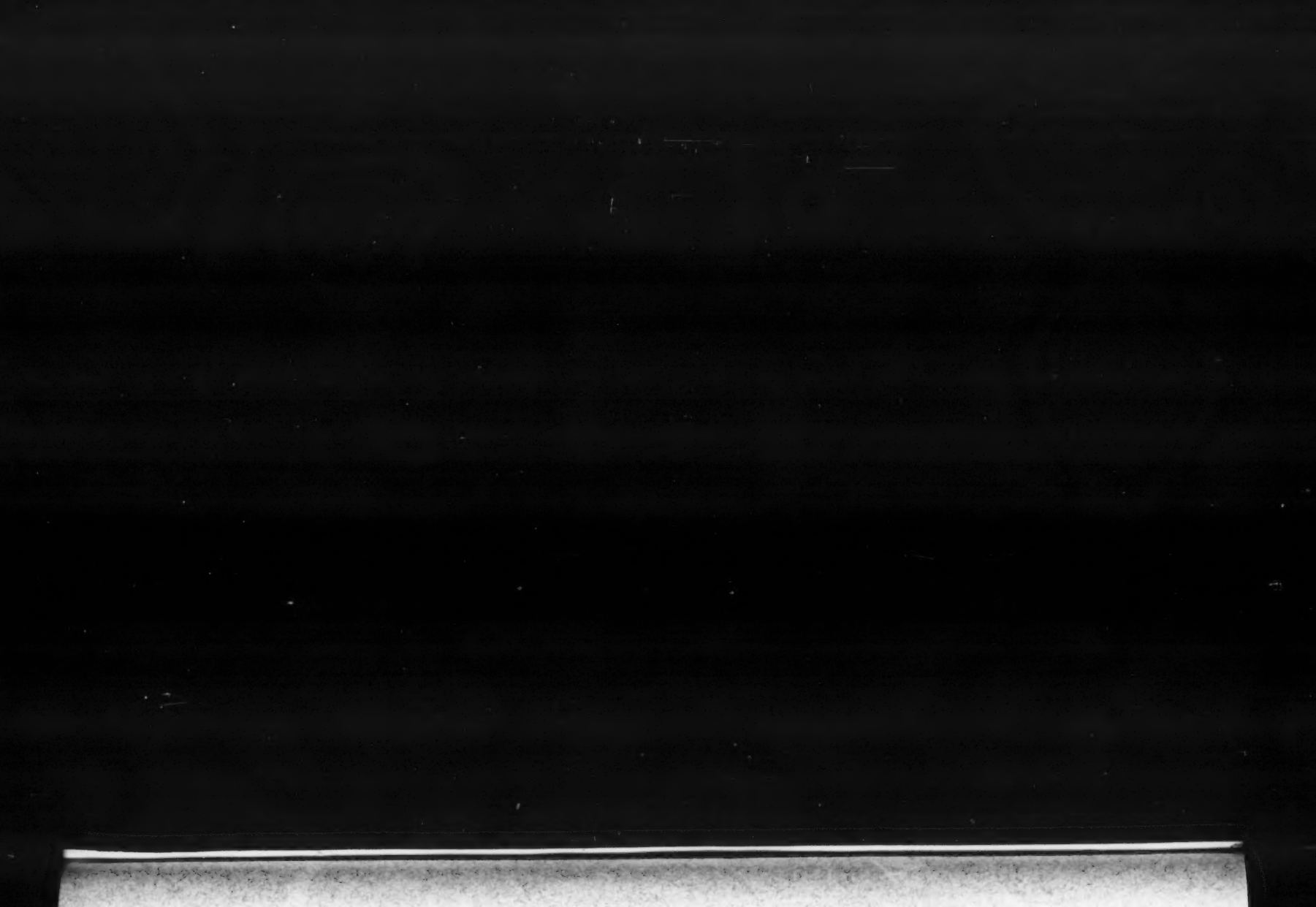

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BIOLOGICAL BULLETIN

AUTHOR'S ABSTRACT OF THIS PAPER ISSUED BY THE BIBLIOGRAPHIC SERVICE, FEBRUARY 7

OBSERVATIONS ON THE DISTRIBUTION AND HABITS OF THE BLIND TEXAN CAVE SALAMANDER, TYPHLOMOLGE RATHBUNI.

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When in 1895 the artesian well was drilled at the U: S. Fish Hatchery in San Marcos, Texas, the first specimens known to biologists of the blind cave salamander, *Typhlomolge rathbuni*, were brought up with the waters from the depths of the ground. The animals were described by Prof. L. Stejneger. For several years after this a relatively large number of the blind salamanders, about 100 a year, were found in the basin of the well, but gradually the number decreased and lately has been reduced to a few specimens a year.

When the question arose of subjecting this animal to certain experiments on metamorphosis, it became evident that a number of specimens sufficiently large for this purpose could be obtained only through an extensive search in the actual habitat of the *Typhlomolge*. With the aid of a special grant from the Rockefeller Institute for Medical Research an extensive study of the caves of San Marcos and environment was made by the writer and Mr. C. A. Campbell, at that time instructor in biology at Coronal Institute in San Marcos, during the months of August and September, 1916. So far as the number of animals obtained is concerned, the result was disappointing. But, on the other hand, several observations were made which seem to be of interest as regards the distribution and habits of the *Typhlomolge*

and which furnish valuable suggestions as to the methods which must be employed in order to procure a large number of animals. The writer hopes to stimulate a search for these salamanders on a large scale, in order to make this interesting form accessible to the experimental biologist who is in need of just such an animal as *Typhlomolge rathbuni* for attacking many important problems.

General Characters of the Region.

As pointed out, the specimens described first by Stejneger were found in the basin of the Artesian Well in the Fish Hatchery in San Marcos, and were carried up into this basin by the flowing water of the Artesian Well. During a two months' stay in San Marcos, we secured only two specimens from this basin, but five other specimens were found in three other localities, *i.e.*, in Frank Johnson's Well, in Ezell's Cave and in Beaver Cave.

In order to understand the conditions which might have led to the present distribution of *Typhlomolge* and because these conditions in the future may be an important guide in tracing the subterranean channels which the animals inhabit, a careful study was undertaken. It was found that the conditions in the three places where we found *Typhlomolge* are essentially similar to those existing in the locality from which the water of the San Marcos Artesian Well is derived.

Before describing the well and the caves in which we found *Typhlomolge* it is necessary to point out the geologic peculiarities of this area of Texas, since these conditions not only led to the formation of the caves but also to the present distribution of the *Typhlomolge*. Whether or not they are also responsible for the peculiar characteristics of the animal as Eigenmann and Stejneger assume, is an important question, the answer to which, however, cannot be given before extensive experiments on this species have been carried out.

San Marcos is located on the so-called Balcones scarp line. This line runs from Austin to Del Rio in a south-westerly direction and separates in a most distinct way the Edwards Plateau (north of the line) from the Rio Grande Plain (south of the line). It forms the escarpments of the plateau towards the plains. Along this line a faulting has taken place in Eocene time (Hill

and Vaughan, p. 260), during which the part that now forms the plain was thrown down and the northern part which now constitutes the plateau was left behind. In consequence of this faulting, any particular geologic stratum now lies deeper on the side thrown down than on the plateau.

It was apparently this faulting which has led to the formation of many cracks in the rock layers. The caves near the escarpments of the Edwards Plateau represent gigantic cracks. Besides this factor there is still another cause leading to the formation of caves in this region. The entire area of the Edwards Plateau constitutes a huge outcrop of the Cretaceous. In the soft strata of the various cretaceous formations of the plateau, numerous caves have been formed by the mechanical force of the water combined with its dissolving action. By this process most of the rivers of the Edwards Plateau have disappeared almost entirely from the surface, and their former beds are dry. These rivers have sunken beneath the surface where they flow in subterranean channels.

The San Marcos Artesian Well.

When the Artesian Well of the U. S. Fish Hatchery in San Marcos (Fig. 1) was drilled in 1895, a number of water reservoirs were opened up by the drill. At present only the water is used which rises from a depth of approximately 190 feet. Here a cave filled with water was opened up; in it the *Typhlomolge* lived. The water in this cave must have been under a pressure sufficiently high to carry it up 190 feet. The *Typhlomolge*, thus, lived most abundantly in water under high pressure and without any access to air except that present in the water. The water of this cave belongs to the so-called "sweet water" horizon of the Edwards limestone in which formation the cave is located.

We measured the temperature of the water as it comes out of the tube of the well as approximately 21.5° C. Among the fauna of the cave from which the water of the San Marcos Well rises, are particularly conspicuous two crustaceans, both unpigmented and eyeless, an isopod, *Cirolanides texensis* and a decapod, *Palæmonetes antrorum*. The latter species is of particular importance,

since so far it has been found to occur in all localities which are inhabited by the blind salamanders.

The cave of the San Marcos Well, thus, is characterized in the following manner: (1) It is situated in the Edwards limestone. (2) It contains water derived from the "sweet water" horizon.

FIG. 1. Basin of the Artesian Well of the U. S. Fish Hatchery in San Marcos.

(3) The temperature of the water is approximately 21.5° C. (4) The water is inhabited by the decapod, *Palæmonetes antrorum*.

FRANK JOHNSON'S WELL.

Approximately two miles southwest of the San Marcos court house (see map, Fig. 2), the flat valley of the dry Purgatory Creek crosses the Balcones scarp line opening here into the flat valley of the San Marcos River. Its northern slopes are formed here by the San Marcos Hill. Purgatory Creek originates near the Devil's Backbone, the divide between the Guadalupe and Blanco Rivers, at Boyett's Farm, about 14 miles northwest of San Marcos. It is dry at present, but several of the older in-

habitants claim that this creek had running water in it until about 50 years ago. At present only a few water holes are left in the upper course of the valley and several sink holes have formed in its lower course. These are filled temporarily with rain water. In time of severe cloud bursts the water in the creek becomes a

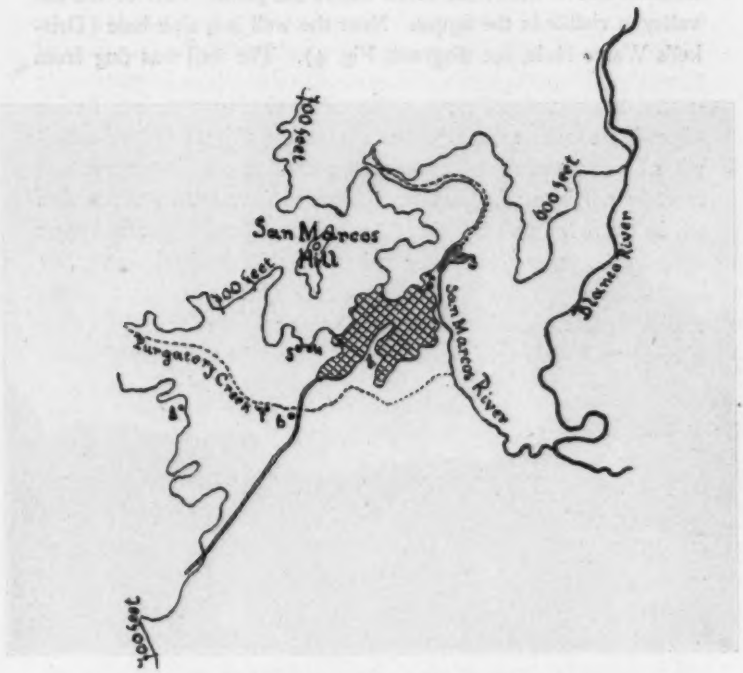

FIG. 2. Map of San Marcos Area.

1. San Marcos.
2. Artesian Well of U. S. Fish Hatchery.
3. Head of San Marcos River.
4. Beaver Cave.
5. Ezell's Cave.
6. Frank Johnson's House.
7. Frank Johnson's Well.
8. Swift's Cave.

torrent rising to a height of 8 feet, but it disappears completely within several hours.[1] Purgatory Creek has now become a subterranean creek. Mr. Frank Johnson's farm is located near where the creek crosses the fault line.

[1] The general character of a creek like this may be found described in Hill and Vaughan, page 207.

Mr. Johnson informed me soon after my arrival in San Marcos that the blind white salamander has been seen in his well, and in fact this well has yielded us more salamanders than any other place. It is shown in Fig. 3.

The well is located in the valley of Purgatory Creek, a short distance above where the creek enters the plain. Part of the flat valley is visible in the figure. Near the well is a sink hole (Driskel's Water Hole, see diagram, Fig. 4). The well was dug from

FIG. 3. Frank Johnson's Well. Showing the well house and at the right of the well house the dry and flat valley of the Purgatory Creek.

a level of 613 feet[1] above sea (San Marcos Court House 620 feet) to a depth of 31⅔ feet. There a cave was struck which now communicates with the well through a slit in the well bottom, as indicated in the diagram (Fig. 4). From this slit the water rose to from 3 to 5 feet in the well. This makes the sur-

[1] Altitudes above sea level were measured by means of an anaeroid barometer and therefore are only approximately correct (within several feet). Dimensions other than altitudes were measured directly, except when otherwise stated.

face altitude of the water about 584 feet. Mr. Johnson claims that the water is flowing. There is no doubt that Johnson's Well communicates with the subterranean Purgatory Creek. As in the case of the San Marcos Artesian Well, the water in this completely water filled cave must have been under a pressure sufficiently high to lift it to 3 feet in the well. It again is evident that the *Typhlomolge* prefer to live in water under high pressure and in caves which are filled entirely with water. The water of Johnson's Well has the same temperature as that of the San Marcos Artesian Well and also has the same taste. Besides the *Typhlomolge,* Frank Johnson's Well contains also the *Palæmonetes antrorum* and the *Cirolanides texensis.*

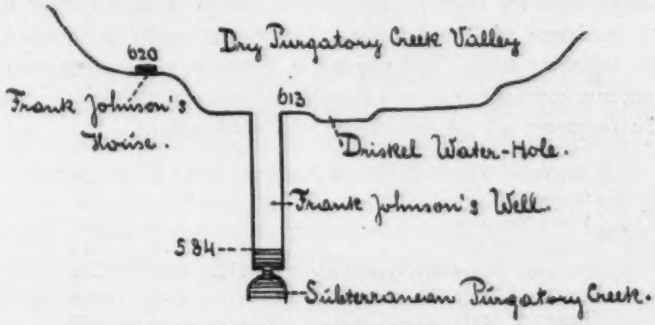

FIG. 4. Purgatory Creek Valley and Frank Johnson's Well. Diagrammatic section reconstructed from several cross sections. The figures indicate altitude above sea level in feet.

Thus, though the water of the Frank Johnson Well represents the subterranean Purgatory Creek, it shows great similarity to the water of the San Marcos Artesian Well. Particularly the presence in Purgatory Creek of 3 species typical of the artesian well would suggest that in some way the Purgatory Creek water is in communication with the so-called sweet water horizon near the San Marcos Artesian Well.

In Frank Johnson's Well the *Typhlomolge* were seen to pass through the slit from the cave into the well. During my stay in San Marcos, the water in the well was too high to catch the salamanders directly and for this reason traps were submerged in the well. These were ordinary minnow traps. In the beginning they

were supplied with various kinds of bait, but in this way only the crustaceans mentioned above were caught. The *Typhlomolge* did not seem to react to the bait, and later on when I observed the animals in the laboratory, it became evident that the instinct of hunger is not sufficiently strong in the *Typhlomolge* to make them go into traps; it is in fact very difficult to make these animals eat. Later on the traps were placed with one opening directly in the slit; animals passing out from the slit had to go directly into the trap. In this way 2 *Typhlomolge* were caught in Johnson's Well, one in August, 1916, and another in September, 1916. After I had left, 11 more *Typhlomolge* were found by Mr. C. A. Campbell and Mr. Rufus Smith who from time to time looked after my traps. Thus, Frank Johnson's Well yielded us 13 specimens of *Typhlomolge*. They were caught as shown in the following table. The number is, however, too small to warrant any conclusions as to a possible influence of the season upon the frequency of the occurrence of *Typhlomolge*.

August,	1916	1
September,	1916	1
November,	1916	2
December,	1916	1
January,	1917	2
April,	1917	1
Summer,	1917	2
November,	1917	3

One of the greatest difficulties encountered was to find a method of shipping the animals from San Marcos to New York; most of them did not survive the trip. In fact, only two ever reached the laboratory alive. The first seven specimens caught were taken on the train in a bucket filled with water. The jarring killed six. Among the eleven caught later on, only one survived the trip. Its safe transfer was accomplished by a fortunate incident. The animal was packed in a fruit preserving jar filled entirely with water and shipped in the winter. On arrival it was frozen tightly in a block of ice. This animal survived for one year in the laboratory. The only thing it could be made to eat were newly hatched larvæ of *Ambystoma maculatum*. Though kept for most of the time in a dark room, the skin which in the

beginning was white with a bluish, mother-of-pearl gleam, had darkened somewhat.

It should be pointed out here that slow reaction to food as exhibited by the *Typhlomolge*[1] is noteworthy in regard to certain findings of Miss E. T. Emmerson, who claims, upon anatomical reasons, a close relationship between *Typhlomolge* and the larvæ of *Eurycea rubra*. We are keeping a large number of such larvæ in the laboratory and contrary to my experience with the larvæ of *Ambystoma* and other salamander larvæ, these larvæ react very slowly to food. In fact, it is impossible to make them eat every day aside from the fact that most of the individuals of this species will eat only at night.

Ezell's Cave.

Ezell's Cave was opened up several years before the San Marcos Well was drilled. The entrance to the cave is located on the southwest slope of the San Marcos Hill (see map, Fig. 2), where it slopes down to the valley of Purgatory Creek about 2 miles W.S.W. of the San Marcos Court House, and not far from a little ravine, the bed of the dry City Boundary Creek, a tributary to Purgatory Creek. This location of Ezell's Cave indicates that it belongs to the Purgatory Creek System, the river found in it probably being the subterranean course of the City Boundary Creek.

Ezell's Cave distinctly exhibits the aspect of a large crack in the strata of the hill, brought about by dislocation of the strata towards the Purgatory Creek Valley. The entrance to the cave (approximately 670 feet above sea level) is part of a 62 ft. slit in the surface (Fig. 5), which for the most part is closed up by large rocks and runs from N.N.W. to S.S.E., that being the direction of the long axis of all the various parts of the cave. As the diagrammatic cross and longitudinal sections (Fig. 6 and 7) indicate, the entire slit so far as accessible is divided into two compartments by means of the rock masses which were thrown down during the process of dislocation and following corrosion.

[1] Normann, who kept a specimen of *Typhlomolge* in captivity, also reports great difficulty in making the animal eat.

These masses of debris form the bottom of the first story and in the N.N.W. corner leave open a small hole ("entrance hole"), 2¼ feet wide through which a narrow canal ("tube") may be reached which after running along the main axis of the slit for a short distance leads down into the second story or water room.

FIG. 5. Entrance to Ezell's Cave.

This compartment of the cave contains a large body of water (Fig. 8).

This pond is not formed by water which drains through the strata forming the roof of the cave nor by water flowing directly into the entrance of the cave, as the slope of the hill is drained in

the course of rain. The pond is formed by a subterranean river, which is evident from the fact that the water is flowing, though hardly in a perceptible manner. The flow can be observed from the dislocation of bodies dropped into the water at the N.N.W. end of the pond. If the water is not disturbed such bodies will arrive, in the course of an hour or so, at the S.S.E. end, thus indicating the direction of the flow. By means of a collapsible

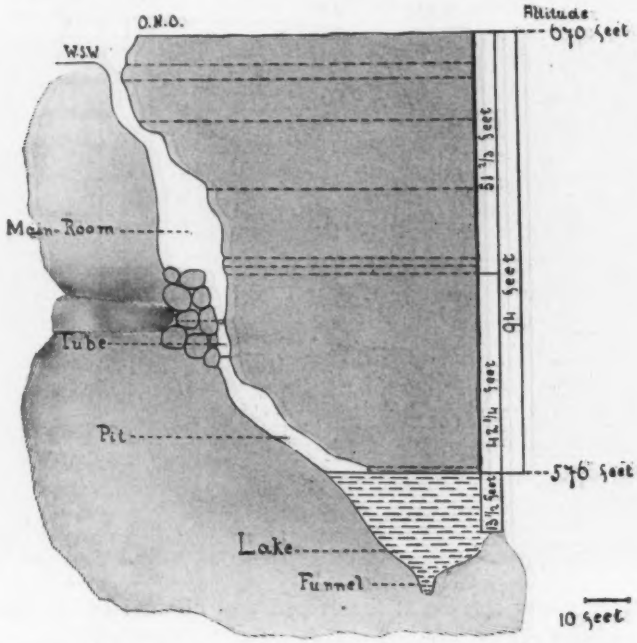

FIG. 6. Ezell's Cave. Diagrammatic section reconstructed from several cross-sections.

boat which was brought down into the water it is possible to follow the course of the subterranean creek towards N.N.W., (Fig. 9) for a distance of about 91⅓ feet. The crack extends, however, beyond this point and by climbing over a number of rocks the creek can be seen to continue in this crack. But we had no opportunity so far to explore this part of the cave.

The greatest depth of the water is 13½ feet, as far as it can

be measured. It is, however, not possible to ascertain exactly the depth of the water and of the crack, since the water is covered in part by the overlapping wall of the crack forming a ledge over the water (diagram Fig. 6 and photograph Fig. 9). Underneath this ledge the ground can be seen to slope down very deeply; it is possible by means of a strong light to see a funnel-

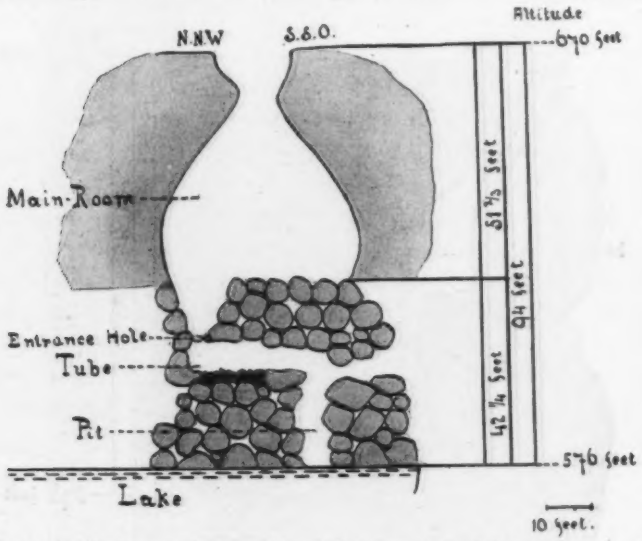

FIG. 7. Ezell's Cave. Diagrammatic section reconstructed from several longitudinal sections.

shaped crater opening at the deepest part of the lake in which no bottom can be seen.

The entire crack, with the water which it contains, is located in the Edwards limestone; but as pointed out above, the structure of the cave would indicate that this crack may extend into the deeper lying strata.

The distance from the entrance down to the water surface is 94 feet, which makes the level of the water about 577 feet. The altitude above sea level of the entrance of the cave was measured merely by means of a barometer, but the figure approaches the altitude of the water surface in Frank Johnson's Well near

enough; the water levels in Frank Johnson's Well and in Ezell's Cave are approximately equally high.

The water is of an extreme clearness and of bluish color, typical also of the water of the sweet water system. It also tastes like this water and has the same temperature (21.5°). Using a sufficiently strong light one discovers immediately a great number of *Palæmonetes antrorum* swimming near the sur-

FIG. 8. Water-room in Ezell's Cave.

face of the water, which thus contains also the same species of animals as were found in the water of the San Marcos Artesian Well and in Frank Johnson's Well.

Hence the water in Frank Johnson's Well and that in Ezell's Cave have a number of characteristics in common. They have the same taste, are of the same temperature, and their levels are equally high. They harbor the same species of animals. From their characteristics and from their location it seems that they are parts of the subterranean Purgatory Creek System.

Furthermore, both of these water bodies have certain most

conspicuous characteristics in common with the water of the San Marcos Artesian Well. They are of the same temperature and contain the same fauna. One naturally would think of a direct communication between the Purgatory Creek System and the caves which supply the San Marcos Artesian Well.

FIG. 9. Ezell's Cave Lake. Showing the overlapping ledge.

We caught only one animal (78.5 mm.) in Ezell's Cave. It was sitting quietly near the bank of the river where the water is shallow, and did not seem to mind pebbles dropped down into the water near it, nor the glare of the light from two Columbia dry cells. We spent 12 days in the cave under the most varied con-

ditions, and conducted a most extensive search for *Typhlomolge*. Hence the scarcity of this species is somewhat perplexing. It is possible that the animals prefer to stay further down in the passages and cracks filled completely with water under high pressure, an assumption which is supported by the circumstances under which the animals were found in both the artesian well and Frank Johnson's Well. It may be that they rarely and only by some incidental circumstances are induced to come to the more open bodies of water.

So far as is known to the writer, the specimen of *Typhlomolge* caught in Ezell's Cave in 1916 is the first and only one positively known as having come from this locality. But it is claimed by people in San Marcos, as Mr. S. N. Stanfield, teacher of biology in the Texas Normal School in San Marcos, informed me, that the first two *Typhlomolge* ever seen were found in Ezell's Cave, 1½ years before the well was drilled, in a small boat which had sunk in Ezell's Cave Lake.

Beaver Cave.

Not far from the entrance of Ezell's Cave on the southwest slope of San Marcos Hill and at an altitude of 652 feet above sea level, near the dry bed of the City Boundary Creek is situated the entrance to Beaver or Wonder Cave. The location of the cave would indicate that it belongs, like Ezell's Cave, to the Purgatory Creek System.

Beaver Cave represents the aspect of a straight running crack in the strata of the Edwards limestone, the same as Ezell's Cave; this crack, in part, has been widened out and its walls have been smoothed down by the action of the water (Fig. 10). Its bottom is made up of huge masses of broken-down rocks which form, at some places, high cliffs and rock masses, dividing the entire cave horizontally in a number of rooms connected by narrower tubes with one another, and vertically into several compartments. Fig. 11 represents a diagrammatic longitudinal section through the cave, which gives an idea of the construction of this cave. In Fig. 10, which was taken parallel to the longitudinal axis, the slit-like shape of the cave is shown; it can also be seen how smooth

the walls have been washed by the water entering easily through the thin roof of the cave.

The longitudinal axis of Beaver Cave runs from N.N.E. to S.S.W., forming an angle of approximately 25° with the longi-

FIG. 10. Interior of Beaver Cave. Photograph taken from rock 34 towards board rock. In back of the right hand side wall at its lower end, the opening of the "tube" is visible.

tudinal axis of Ezell's Cave; the length of the entire slit is nearly 500 feet. It is claimed that there is a direct connection between Beaver Cave and Ezell's Cave. We could not verify this statement, and it seems certain no one has actually found a connection. We found that at *x* in room VI. (see Fig. 11) a number of

tightly packed rocks and masses of gravel make further penetration impossible at present and that the location of both caves and the direction of their main axes are not in favor of the statement mentioned above.

The deepest depression in the bottom of Beaver Cave is found in the room indicated in the diagram Fig. 11 as "Well-Room." The bottom of this floor is 62 feet below the surface and therefore at a level of 590 feet above sea level. As seen from the height of the water level in Johnson's Well and in Ezell's Cave, no water of the Purgatory Creek System should be present in Beaver Cave. And in fact when the cave was discovered there was no water found. But a well drilling made at that time from the surface above the Well Room had indicated the presence of water only a few feet beneath the bottom of the Well Room. Therefore, a hole was dug in the bottom of the Well Room which led to water at a depth of about 3 feet or at the same level as the surface of the water in Ezell's Cave and Frank Johnson's Well (see Fig. 13).

At present one finds in the Well Room of Beaver Cave a rectangular basin approximately 6 feeet in length, 3 feet in width and 6 feet in depth, the bottom of which is covered with mud and rocks, and the walls of which are lined with logs.

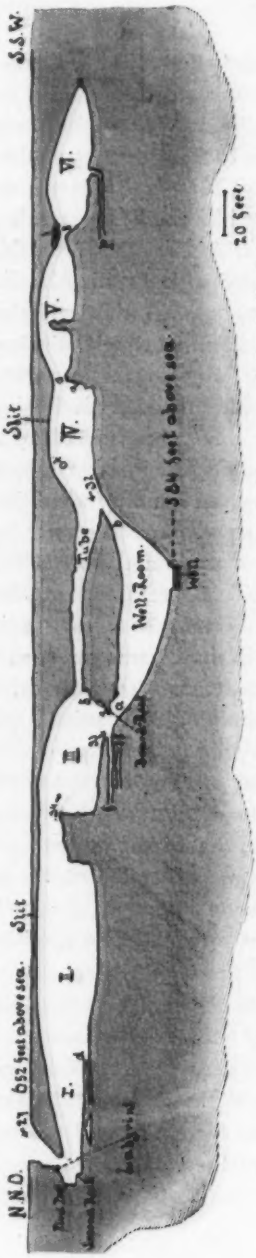

FIG. 11. Beaver Cave. Diagrammatic section reconstructed from several longitudinal sections.

This basin is filled with water half of its depth. Hence the surface of the water stands at the same level with the surface of the water in Frank Johnson's Well, and the suggestion seems justified that in this basin again part of the Purgatory Creek System was opened up. The water has the same taste as the water of the other localities mentioned and also has the same temperature (21.5° C.). In which way, however, this basin in Beaver Cave could be connected with the other localities cannot be stated with certainty at present, since the log lining of the wall made it impossible to search more closely whether or not the rocks of the wall contain any larger cracks or crevices. It also was not determined whether the water is flowing. But its clearness and the fact that the mud when stirred up disappears in a relatively short time would speak in favor of a slight current in the water. There is, however, one fact which hardly could be explained in any other way than that the water in the basin must be in connection at least at certain times with some larger bodies of water. The well in Beaver Cave contains both the *Palæmonetes antrorum* and the *Cirolanides texensis,* animals the transmission of which to the basin since it was constructed must have taken place by means of water currents which drive water from certain water bodies (harboring these animals) through the well.

Hence it is most probable that the water of Frank Johnson's Well, of Ezell's Cave and of Beaver Cave is the water of the subterranean Purgatory Creek System.

In the well of Beaver Cave two *Typhlomolge* were caught, one by means of a dip-net, the other in a trap which was laid with its opening just in front of a hole into which the animal had been seen to pass. One specimen was 82 mm. in length, the other one the largest caught measured 120 mm. Both these animals were observed for some time before they were actually caught; they proceeded to move in characteristic fashion—as described very accurately by Normann—by intermittent walking and resting in the presence of light. Even when the rays fell directly upon them, they did not seem to be disturbed. In this respect our observations made in the animal's natural habitats, agree very well with the observations made by Normann in the labora-

tory. Pebbles and a pocket-knife dropped into the water near the animal did not change its behavior; we have not found that the *Typhlomolge* as Normann claims possesses a specially high sensitivity towards disturbances of the water. Once stirred up the animals immediately swim towards the walls, and if they cannot find cover immediately, they swim along the wall toward the surface pushing out their snouts above the surface.

Before I was acquainted well enough with the general situation in the localities in question and before I had other facts indicating a possible connection between Beaver Cave and the Purgatory Creek, the occurrence of the *Typhlomolge* in the Beaver Cave well was puzzling, since it seemed to be difficult to explain how they could have been transferred to the well. In an anatomical study performed on *Typhlomolge rathbuni,* E. T. Emmerson points out the close relationship existing between *Typhlomolge* and *Eurycea* (*Spelerpes*), in particular *Eurycea rubra* and suggests that *Typhlomolge* may be the larva of an unknown species of the genus *Eurycea.* The writer of this article has a large number of larvæ of *Eurycea rubra* under observation and finds that in certain habits (feeding and especially the pushing out of the snout above the water when aroused) a remarkable resemblance exists between *Typhlomolge* and *Eurycea rubra,* a resemblance which was not observed by the writer in larvæ of the many other species of salamander closely watched in the laboratory. Concerning, however, the assumption that *Typhlomolge* is the larva of some species of *Eurycea,* this meets with one difficulty if it should mean that this species is still in existence. Ezell's Cave and especially Beaver Cave were closely searched for the presence of other salamanders. None were found in Ezell's Cave. In Beaver Cave, however, Mr. Campbell found about 20 specimens all belonging to the species *Plethodon glutinosus;* this is the only salamander which we could detect in these and other caves of the area around San Marcos. In view of this fact it appears that the suggestion as to whether or not *Typhlomolge* is the larva of a species represented at the present time also by metamorphosed specimens would be hardly more than speculation. It is, however, certain that it would be of the

greatest value to raise the *Typhlomolge*, in order to study closely their mode of propagation, development and to subject these animals to certain experiments indicated by our present technic in the study of the metamorphosis of other salamanders.

In connection with the metamorphosis of *Typhlomolge* it may be pointed out that Miss Emmerson has made a statement which is so important that it arouses curiosity as to why it has attracted so little attention. Miss Emmerson searching for the organs of internal secretion of *Typhlomolge* found that the animal possesses a thymus gland, but she could not find a thyroid gland. If the lack of a thyroid gland could be confirmed—and we are preparing some of our specimens for examination with that end in view—Miss Emmerson's discovery will explain why the *Typhlomolge* cannot metamorphose at present, since Allen has demonstrated that larvæ of frogs and toads whose thyroids were extirpated did not metamorphose, though the controls with intact thyroids all metamorphosed. Do the Proteidæ (*Proteus*) possess thyroids, is the lack of the gland common to all of them? And what are the reasons for the atrophy of the gland? These are problems which call urgently for investigation.

From the facts mentioned above it is certain that the *Typhlomolge* inhabit the subterranean waters which constitute the Purgatory Creek System and a subterranean water channel which supplies the San Marcos Artesian Well. These two systems are located north and south respectively from the Balcones scarp line. On account of the faulting, though both the Purgatory Creek Caves and the Artesian Well Cave are located in the same geological formation, the latter cave occupies a position several hundred feet deeper than the Purgatory Creek Cave; this is indicated in the diagram, Fig. 12. The water in both systems is of different origin, as may be seen from this diagram. The water of the Artesian Well is the so-called "sweet water," which on the plateau, *i.e.*, in the region of the Purgatory Creek System, is carried in beds below those in which the caves of the Purgatory Creek System are located. The "sweet water" is caught by the basement beds of the Cretaceous, the Travis Peak and Glen Rose formation, where they outcrop on the plateau, and is carried

down along the slanting stratum beneath the geologically higher situated Edwards limestone and towards the fault. Along the fault, however, the continuity of the water-bearing strata is broken and they come to lie in one level with the Edwards limestone of the plain; thus, here the water is forced from the Glen Rose formation into the Edwards limestone.[1]

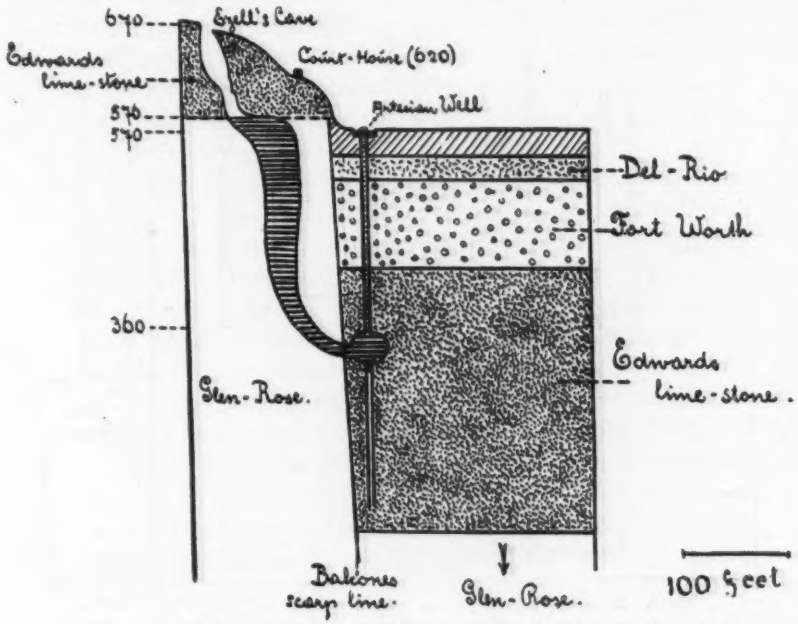

FIG. 12. Ezell's Cave. Balcones scarp line and Artesian Well of U. S. Fish Hatchery in San Marcos, Texas. Diagrammatic section showing the position of northern and southern part of the various cretaceous formations to each other after the dislocation of the Rio Grande Plain in Eocene time accomplished by faulting. The figures on the left-hand side of the diagram indicate altitude above sea level in feet.

The water of Johnson's Well, Ezell's Cave and Beaver Cave, however, is the river water of the subterranean Purgatory Creek. The level of the Purgatory Creek in these localities at present is at an altitude of about 580 feet, that of the Artesian Well 360 feet.

[1] Hill and Vaughan, page 315.

But as indicated above, it is quite probable that a direct communication has been established between these two systems by means of channels which according to our calculations would have a depth of about 200 feet; if this is a fact, the relation between the various bodies of water in question would be as shown in the diagram of Fig. 13.

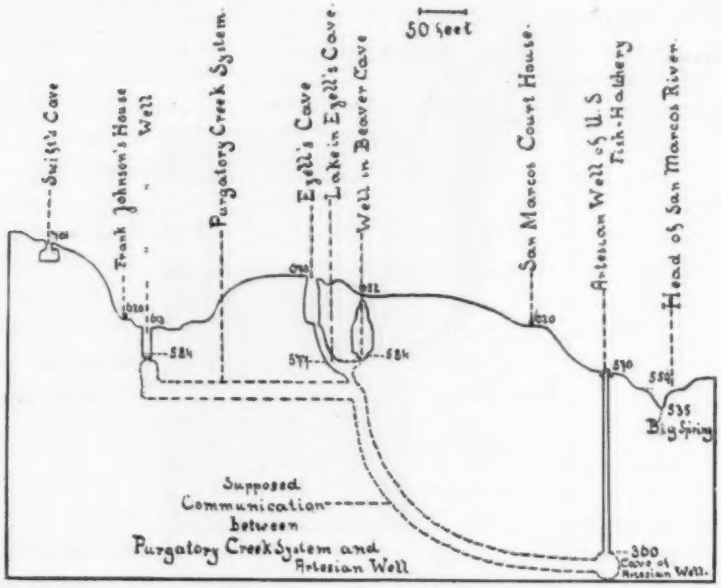

FIG. 13. Purgatory Creek System. Artesian Well and San Marcos Springs at San Marcos, Texas. Diagrammatic section reconstructed from several sections, showing the way in which these waters probably communicate with each other. The figures indicate altitude above sea in feet.

It is of great importance to ascertain whether or not such a communication exists, since this would facilitate following the *Typhlomolge* along the course of travel and since it would permit conclusions as to the mode of the distribution of the species. Besides the suggestive structure of Ezell's Cave there are a number of facts which are in favor of the existence of a communication. If no connection between the two systems exists it would mean that the *Typhlomolge* lived in the subterranean rivers be-

fore the present southern and northern parts of the Edwards limestone were separated from each other, and that after the dislocation in Eocene time part of the species was caught in the caves of the Edwards limestone of the San Marcos area south of the Balcones where it lived completely isolated from the rest of the species. Since the specimens obtained from Ezell's Cave and the Artesian Well are identical, it would mean either that the species remained absolutely unchanged since Eocene time, or if it changed, underwent exactly similar changes in the open ponds of the subterranean Purgatory Creek and in the completely closed and water-filled subterranean caves of the Artesian Well. It is evident that none of these possibilities is probable.

Not only the Artesian Well at San Marcos but numerous other artesian wells along the Balcones escarpments are supplied from the sweet water horizon; yet from none of them, except the San Marcos Well, *Typhlomolge* has ever been reported. This would be explained if the San Marcos Well contains besides the sweet water also the Purgatory Creek water, since this certainly could not be true for the other wells. Probably the Purgatory Creek is the original habitat of the *Typhlomolge* and later on the animals migrated down to the water channels of the Artesian Well.

Also in none of the fissure springs of the Balcones scarp line, not even in the San Marcos springs though they all come from the sweet water reservoirs, *Typhlomolge* ever has been collected. The same explanation as to the artesian wells could be applied to these springs, if a communication exists between the Purgatory Creek System and San Marcos well.

Finally an incident may be mentioned here which also would speak in favor of the existence of a direct communication between the Artesian Well and the Purgatory Creek System. Mr. Mark Riley, superintendent of the U. S. Fish Hatchery, informed me that in the basin of the Artesian Well a number of catfish were kept at one time, but they disappeared gradually from the basin and it is claimed that they migrated into the tube of the artesian well. The writer is not prepared to form an opinion concerning the probability of such migration. One day, however, while I was looking for *Typhlomolge* in Ezell's Cave, I saw some fishes

hiding behind the rocks. Shortly after this we caught two fishes by means of hooks which were placed near the rocks where I had seen the fishes; both were catfish. And they were the only specimens of fish which I ever saw in Ezell's Cave during the 12 days I spent there. If these were identical with the individuals kept in the basin of the Artesian Well, it certainly would be proof of the existence of a communication between the Purgatory Creek System and the San Marcos Artesian Well. It would be of great importance to trace the course of the water in Ezell's Cave and Johnson's Well down to the reservoir of the Artesian Well. As suggested by the possible migration of the catfish, such methods could be easily designed and will be employed as soon as the investigations can be continued.

In case of a connection between the two systems, the water contained in each one would be a mixture of the Purgatory Creek water and the sweet water. In all four places in question the water has the same taste and the same temperature. It contains besides the *Typhlomolge* a number of typical species, among them the *Palæmonetes antrorum*, which I found to occur in all four localities.

Other Localities in Purgatory Creek Valley.

After it was found that the *Typhlomolge* inhabit subterranean regions probably representing the Purgatory Creek System, it was interesting to visit other caves of the Purgatory Creek. One of them is Swift's Cave, on the slope closing the valley towards southwest and abuot 1 mile above Frank Johnson's Well. The entrance to the cave is situated at an altitude of 701 feet above sea level. Though no water could be reached so far—according to what has been said above, it would have to be found about 114 feet below the entrance—there is in this cave a narrow tube leading down which has not been followed; further examination may reveal the presence of some passages to the water.

Further up the valley 14 miles above San Marcos, Boyett's Cave is located at an altitude of about 1,100 feet; there the Purgatory Creek valley starts. No water was found in Boyett's Cave down to a depth of 50 feet and no passages leading further

down were discovered. But in the large main hall of this cave one notices along the walls a whitish deposit for about 3½ feet above the ground and forming a straight horizontal line running along the walls, as seen in Fig. 14. This indicates the former presence of water in this cave. Probably with the general dis-

FIG. 14. Boyett's Cave.

appearance of the water from the Purgatory Creek valley and its fall to deeper levels, flowing water has disappeared from the cave. There are, however, a number of small shallow pools (several inches deep) formed from dripping water in the sandy bottom

of the main hall. In these pools certain crustaceans are found in large numbers, which according to Dr. Ortmann, are at least very closely related to if not identical with the species *Stygonectes flagellatus*,[1] an amphipod known from the San Marcos Well. Thus, this animal, which through its mode of living is well adapted to the conditions prevailing at present in Boyett's Cave, is the only remnant there of the Purgatory Creek System fauna.

The San Marcos Springs.

According to Hill and Vaughan it is quite certain that the San Marcos Springs, like all other fissure springs along the escarpments of the Edwards Plateau, are of the same origin as the artesian wells of this area, and hence the water of the San Marcos Springs comes from the same reservoir which supplies the Artesian Well at San Marcos Fish Hatchery. We might expect therefore that between these two localities ways of communication exist along which the *Typhlomolge* may travel.

We have not so far subjected the San Marcos Springs to a thorough examination, but a brief mention may be made of certain facts valuable for future exploration. The water of the San Marcos Springs comes from funnel-like depressions of the surface (see diagram, Fig. 11), and forms a little lake which is the head of the San Marcos River. The openings through which the water emerges lie deeper (at an altitude of only 532 feet) than the surface of the water of Purgatory Creek. The surface of the lake which is artificially dammed, is 559 feet above sea level. The temperature of the water in a little spring on the bank of the lake is 21.5° C., like that of the water of the Purgatory Creek system and the Artesian Well.

Towards the south the valley at the head of the River continues and forms the bed of the San Marcos River in which the water is flowing, but this valley can be traced also north of the springs, though here it is dry.

Unconfirmed claims have been made that the "white salamander" was seen at the head of the river, but that it had developed eyes and turned brownish. These statements are, no

[1] For further information see Benedict and Weckel.

doubt, due to the occurrence there of the larvæ of other salamanders which have been mistaken by the layman for *Typhlomolge*. But even if the animal should come up into the lake, it would be quite difficult to find it there; unaccustomed to such rapacious enemies as certain fishes which abound in the lake, the blind *Typhlomolge* would soon fall a victim.

There are, however, two localities further up in the dry valley which it might be important to examine. One is a hole resembling a well hole because of its regularity. It is about 6 feet deep. Mr. Bidler who is well acquainted with conditions as they were 20 to 30 years ago in this area, informed the writer that at one time this hole was much deeper and contained a small body of water. He assured me that one day in the water of the hole two white *Typhlomolge* were seen. At any rate this hole should be prepared for examination by removing the gravel and rocks and thus penetrating to the water, the level of which would be approximately that of the water in Purgatory Creek. The place could be easily prepared so as to make trapping there a success.

Near this place on one of the slopes of the valley is situated another hole (on the property of Mr. Mark Riley), which much resembles the entrance to Beaver Cave. In former years, before the water which abundantly drains into that hole had washed gravel into it, one could penertate it for some distance and reach a place at which the sound of water could be heard.

The writer believes that the exploration of the two places mentioned would lead to a definite knowledge about the presence of *Typhlomolge* in the San Marcos River valley.

Other Places to be Explored.

It is clear that *Typhlomolge* cannot be procured in abundant number by collecting in one or two places only, since these animals pass only in small numbers from the deep water-filled caves into the open water bodies of the higher horizons. Successful collecting must have as a basis the discovery of a large number of places where some of these animals can be found. Traps must be laid in all these places and watched for some time. Assuming that from three such localities 5 specimens could be obtained in

the course of two months, as was the case in Ezell's Cave, Beaver Cave and Johnson's Well, a steady and skillful worker could collect from 18 such places 180 specimens in a year, a number sufficiently large to start experimental work on the species and to keep a sufficient number for breeding stock. For this reason an attempt will be made to mention briefly a number of other places where *Typhlomolge* may possibly be found.

There are two caves on the ranch of Mr. Bender at Spring Branch, 40 miles above San Marcos and about 1,100 feet above sea level. One is a narrow channel through which the head water of Spring Branch Creek passes out. The channel is filled almost to the top with water but it is possible to penetrate it to a depth of 350 feet. Since the water is flowing quite rapidly, it is not likely to contain *Typhlomolge*, but a more thorough search might be conducted. The other cave represents a narrow crack in the strata containing water at a depth of 45 feet. It is only a small pool, which, however, is part of a larger body of water covered by overlapping ledges. The temperature of the water is 20.5° C. Besides frogs, some other animals inhabit this pool. They could not, however, be identified, as upon our approach they immediately dived underneath the ledge.

More important still is the water on Mr. Bremer's ranch, at the water hole of the Cypress Fork in Hays County, a tributary branch of the Blanco River, about 1,000 feet above sea level. The water hole (Jacob's well) itself is filled with blue water which has a temperature of 22.5° C. On account of the large black basses inhabiting the hole one would not expect to find *Typhlomolge* there. But further up on one of the slopes of the dry valley is located the entrance to a cave in which the water (probably of Jacob's well) could be reached. We penetrated to a place where a number of small holes perforate the bottom of the cave; pebbles thrown into the holes evoked the sound of rather deep water. By dislocating a large rock, it would be possible to make one of the holes large enough to gain access to the water.

It might be valuable to mention a few places in which, according to Mr. S. A. Stanfield, *Typhlomolge* have been seen:
Burnet Cave, Kendall County, near Burnet.

A spring near Twin Sister Mountain, Hays County, 2 miles from Wimberly.

A spring near Ozona, 100 miles from San Marcos.

Summary.

1. At present it is certain that *Typhlomolge rathbuni* inhabits the subterranean water of the Purgatory Creek System just north of the Balcones scarp line and one mile further up, and the caves of the Artesian Well of the U. S. Fish Hatchery at San Marcos, which seem to be in direct communication with the Purgatory Creek System by means of channels about 200 feet deep.

2. The populations of the species *Typhlomolge rathbuni* north and south of the present Balcones scarp line have not been separated from each other by the process of faulting in Eocene time, but have developed in unrestricted communication with one another.

3. No certain data are available as regards the occurrence of *Typhlomolge* in the San Marcos Springs and in the dry valley of the San Marcos River north of the Springs. Since the Springs come from the same water reservoir as the Artesian Well, further investigations should be conducted.

4. All the localities containing *Typhlomolge* are located in the Edwards limestone region, but the caves of the Artesian Well are 200 feet deeper than the rest.

5. *Typhlomolge* have been found in the Purgatory Creek System at an elevation of approximately 585 feet above sea level. Where this level could not be reached as in the upper Purgatory Creek valley, only remnants of the Purgatory Creek System fauna (*Stygonectes flagellatum*) were found.

6. The water inhabited by *Typhlomolge* seems to be slowly flowing water.

7. The temperature of the water is approximately 21.5° C. and it is inhabited by the decapod *Palæmonetes antrorum*. Since the latter animal is much more numerous and can be detected much easier than the *Typhlomolge*, its presence may be taken as an indication that the place is promising as regards the presence of *Typhlomolge*.

8. The rarity of the *Typhlomolge* seems to be due to the animal's habit of preferring deep lying cracks or crevices, completely filled with water at a higher pressure than exists in the more open bodies of water located at higher levels.

9. As regards the habits of the *Typhlomolge* in its natural habitat we were able to confirm Normann's observations made in the laboratory in respect to the peculiar mode of walking of this animal and its indifferent attitude to light. But we did not find the animal particularly sensitive to water waves.

10. In feeding and swimming when aroused, *Typhlomolge* shows a close resemblance to larvæ of *Eurycea rubra*.

11. The assumption, however, that *Typhlomolge* is the larva of some unknown and still existing species of the genus *Eurycea* as made by Emmerson could not be confirmed, since with the exception of the species *Plethodon glutinosus* no tailed Batrachians were found in the caves. More important than this assumption is the fact that *Typhlomolge*, according to Emmerson, lack a thyroid, which would explain why these animals cannot metamorphose.

12. In order to collect a large number of specimens necessary for experimental work and intensive study of the species, as many places as possible must be discovered which may contain *Typhlomolge*, and collecting must be conducted simultaneously in all these places.

13. The best method of catching the animals is by trapping, but this method must be improved. It seems probable that live bait is not attractive to the animals. Instead of relying upon bait, the large openings of the traps should be laid in the path of the animals.

I desire to express my indebtedness and warm thanks for the assistance which they have so generously rendered me in this work, to the persons whose names I take pleasure in stating below:

Mr. C. A. Campbell, instructor in biology at Coronal Institute in San Marcos, for his enthusiastic and skillful assistance and his most enjoyable company during the collecting trips.

Dr. H. F. Moore, Acting Commissioner of U. S. Fish Hatch-

eries, for permission to keep apparatus and outfit as well as material collected at the U. S. Fish Hatchery in San Marcos.

Mr. Frank Johnson for permission to use and abuse his well in every conceivable way and for valuable suggestions and help in catching *Typhlomolge* out of the well.

Mr. Mark Riley, Superintendent of U. S. Fish Hatchery in San Marcos for aiding the work in every possible way.

Dr. W. T. Vaughan, of the U. S. Geol. Survey, and Prof. C. Eigenmann for valuable suggestions as to traveling and local conditions in Texas.

Dr. T. W. Stanton and Mr. L. W. Stephenson, of the U. S. Geol. Survey, for determination of the various rock specimens collected from the caves.

Dr. H. E. Ortmann for identification of the various species of Crustaceans.

Dr. L. Stejneger for identification of the salamander *Plethodon glutinosus*.

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ON THE DEVELOPMENT OF THE SPONTANEOUSLY PARTHENOGENETIC EGGS OF ASTERINA (PATIRIA) MINIATA.

H. H. NEWMAN.[1]

Introduction.

While engaged in a series of experiments on echinoderm hybridology, which I was conducting during the months of April and May, 1920, at Pacific Grove, California, I was forcibly struck by the frequency with which spontaneous parthenogenesis occurs in the starfish, *Asterina* (*Patiria*) *minata*. When I use the term "spontaneous," I mean that eggs were in no way treated either by physical or by chemical agents. Precautions were taken, moreover, to prevent accidental fertilization. The procedure was as follows:

Sea water, brought in from the open sea and therefore free from the chemical impurities present in sea water that has been pumped through metal pipes, was allowed to stand at least four days at laboratory temperatures, which during the month of May ranged from 16° to 19° C. It is certain that no sperms could live for this length of time in sea water. This method is chosen in preference to Loeb's practise of heating the sea water to 60° C. for some time, because it involves no possible chemical changes in the sea water nor any driving out of oxygen. Before opening a starfish, it was scrubbed thoroughly in cold running fresh water and rinsed in a strong stream of fresh water. In case the animal proved to be a male it was discarded, and hands and instruments were scrubbed in fresh water before touching another starfish. If the animal proved to be a ripe female the ovaries were gently shaken into a finger-bowl containing 150 c.c. of the sea water prepared for the purpose and the bowl was covered with a clean glass plate and placed upon a table out of reach

[1] From the Hopkins Marine Station of Leland Stanford, Jr. University and the Hull Zoölogical Laboratory of the University of Chicago.

of direct sunlight. For purposes of observation eggs were removed from time to time and placed in watch-glasses with a sterilized pipette. It was thought safer not to run, as direct controls, normally fertilized eggs, but on other days and under identical conditions numerous observations were made on the course of normal fertilization and development. The differences observed between the behavior of parthenogenetic and that of fertilized eggs were so striking that it seemed well worth while to study and to describe them.

Peculiarities of the Material Used.

Asterina (*Patiria*) *miniata* is one of the commonest starfishes of the California coast. It is a relatively small species in which the five rays are almost completely amalgamated with the central disc in such a way as to give the creature a nearly pentagonal outline. In color it ranges from a brilliant scarlet to a light cream color with various intergrades and piebald combinations. It seems likely that there are several subspecies that freely interbreed. My experience seems to indicate that relatively few eggs mature at a time and are exuded in small numbers over a long season. Among the very large number of females examined I never found an ovary that showed any large percentage of ripe eggs. Those that were shed from the removed ovary and gently shaken in sea water contained oöcytes in all stages of development, some quite small, others fully grown but incapable of maturation, still others mature and ready for maturation and fertilization after standing about one and a half to two hours in sea water. None of the eggs when freshly shed had undergone maturation. This may mean that the artificial shedding of the eggs is a premature process, and that, as seems to be the case in some asteroids, if the eggs were to be normally extruded through the genital pores, they would be immediately ready for fertilization. I have never been able to observe the extrusion of eggs in *Asterina*, nor to induce it by massaging, as can be done in some asteroids. This may possibly be due to some peculiar sexual rhythm in this species, which results in ovulation occurring only at night or at some particular phase of the moon or of the tide.

On this point I have no evidence. So it should be borne in mind that the eggs used in these experiments, and presumably by Loeb, who did a considerable amount of work upon artificial parthenogenesis in this species, are not the normally shed eggs equivalent to those which are fertilized in nature, but are probably prematurely shed eggs. It is very questionable, therefore, whether parthenogenesis ever occurs in nature. It seems more probable that the parthenogenetic eggs observed in these experiments result from the artificial conditions involved in shedding some of the eggs prematurely.

The eggs of *Asterina* are very hardy and resistant of the cytolytic action of sea water. While the unmaturated oöcytes of most echinoderms begin to disintegrate within twenty-four hours, those of *Asterina* frequently remain unchanged, as though in stable equilibrium, for from four to eight days. I have before me a number of microscopic whole mounts, showing numbers of eggs and larvæ of *Asterina* fixed in Bouin's solution on the eighth day after fertilization, in which there occur a number of unmatured oöcytes with germinal vesicle clean-cut and spherical and plasmosome well defined. This ability of the unripe eggs to withstand disintegration is of great practical value in the study of development, for the water is kept free from the products of egg decay; a decided advantage in view of the fact that where there are small percentages of developing eggs surrounded by large percentages of non-developing eggs, the former would have very small chance of survival if the latter were to decay and foul the water.

Experimental Data.

Most of the detailed data on pathenogenesis in *Asterina* were obtained during the month of May, 1920, although the phenomenon was noted incidentally throughout April. The month of May seems to be the best month for work with *Asterina* as there appear to be larger ovaries and more full-grown oöcytes then than earlier. Possibly June would be still better, though I have not tried any experiments at that time.

Some thirty-two experiments were made in all, and no two gave exactly identical results. A large series of ten experiments

made on May 20 shows the full range of diversity and will serve to illustrate all of the points of interest. As the time element is of prime importance in this study it is necessary to give readers as a norm for purposes of comparison a time schedule of the development of the normally fertilized *Asterina*.

TIME SCHEDULE OF THE DEVELOPMENT OF NORMALLY FERTILIZED EGGS OF ASTERINA.

Hours after Shedding.	Condition of Maturated and Fertilized Eggs and Embryos.
2¼	Fertilization membranes formed on majority of matured eggs.
3½	Cleavage beginning.
5	Cleavage taking place in all fertilized eggs, a few 4 and 8 cell stages.
7	Many stages as advanced at 32 + cells.
9	Most of eggs in early blastula stages, but there are a good many eggs without membranes, in early cleavage stages. These are probably parthenogenetic.
25	Two distinct types of larvæ present: the great majority being typical gastrulæ, swimming up near the surface of the dish, and capable of being readily pipetted off; a small minority of living larvæ variously abnormal and swimming at or near the bottom. These latter are probably, though not certainly, parthenogenetic.
49	Normal larvæ, early bipennariæ, forming enterocoel pouches.
73	Bipennariæ with well-defined ciliated bands.
96	Advanced bipennariæ with mouth, œsophagus, stomach, intestine, anterior and posterior enterocœls, waterpore, etc.

TIME SCHEDULE OF THE DEVELOPMENT OF PARTHENOGENETIC EGGS OF ASTERINA.

No. of Experiment.	Hours after Shedding.	Condition of Maturated Eggs or of Developing Embryos.
I	7	No membranes and no cleavage.
	9	About 1 per cent. of eggs show distinct membranes, no cleavage.
	24	About ½ of 1 per cent. swimming blastulæ, all subnormal in appearance, irregular in shape or solid. About 2 per cent. early cleavage stages (2 and 4 cell stages).
	48	All larvæ and cleavage stages undergoing cytolysis.
II	7	No membranes and no cleavage.
	9	No membranes and no cleavage.
	24	No larvæ nor cleavage.
III	6½	No membranes and no cleavage.
	8¾	No membranes, but about 1½ per cent. of cleavage stages, mostly fairly regular 2 and 4 cell stages.
	25	Nearly 1 per cent. blastulæ, mostly solid and motionless.

IV..... 6.....No membranes and no cleavage.
8½.....About 2 per cent. of eggs cleaving without membrane formation, some as advanced as 8 cells.
25.....Nearly 2 per cent. blastulæ, slightly abnormal and motionless.

V..... 6½.....About 5 per cent. of eggs with rather narrow but distinct membranes, no cleavage.
7½.....About 1½ per cent. of eggs without membranes in early cleavage stages (2, 3, 4 cells), some quite regular, but the majority more or less irregular.
8½.....About 5 per cent. of eggs cleaving, ranging from 2 to 16 cells. Evidently a good deal of cleavage has begun since the 7½ hour observation. A few of the eggs now show wide typical membranes and there are transitional stages between these and the more plentiful type with narrow membranes.
26.....About 7 per cent. swimming blastulæ, some nearly normal, but the majority solid, wrinkled or otherwise abnormal. Eggs with membranes, undergoing black cytolysis.
31.....Many gastrulæ, some with two or more archentera some exogastrulæ, etc.
74.....A good collection of twin larvæ, studied in a subsequent connection. No normal larvæ. All larvæ swimming on the bottom of the dish.

VI..... 6¾.....About 4 per cent. of eggs with distinct but narrow membranes, no cleavage.
8¼.....About 2½ per cent. of eggs with wide, typical membranes, but no egg with membrane shows cleavage; about ½ of 1 per cent. of eggs showing cleavage stages, ranging from 2 to 8 cells, some quite regular.
26½.....A very few larvæ, all subnormal, some motionless, others feebly swimming.

VII..... 7.....No membranes, no cleavage.
9.....No membranes, but about 2 per cent. of eggs in cleavage stages ranging from 2 to 32 cells.
26½.....About 1 per cent. subnormal blastulæ, a few swimming.
32.....A very few larvæ undergoing gastrulation; several with two or more archentera.

VIII..... 6½.....No membranes, no cleavage.
9.....No membranes, but about 3½ per cent. of eggs in cleavage stages, ranging from 2 to 16 cells.
26.....About 2½ per cent. of larvæ, all subnormal.

IX..... 7.....About 8 per cent. of maturated eggs with narrow membranes; about 3 per cent. of eggs without membranes showing first steps in cleavage, a few having completed the first cleavage.

8½.....A few eggs in 4- and 8-cell stages.

30½.....A fraction of 1 per cent. of larvæ undergoing gastrulation, and swimming about, the best of them being nearly normal in appearance.

78.....All larvæ dead.

X..... 7¼.....About 5 per cent. of eggs with fully typical membranes, no cleavage stages.

9½.....Over 50 per cent. of maturated eggs in cleavage stages, without membranes, ranging from 2 to 8 cells. No eggs with membranes segmenting.

31.....About 75 per cent. of all maturated eggs have undergone cleavage without membrane formation, and are in various stages ranging from early cleavage to gastrulæ. Numerous dwarf blastulæ, due to blastolomy; many gastrulæ with plural archentera; solid blastulæ and exogastrulæ in considerable numbers. All were swimming about on the bottom of the bowl. This culture was made the basis of a study of the more advanced development of parthenogenetic eggs and will be referred to in more detail in a subsequent discussion.

Significant Points brought out by the Data Shown in the Above Schedule.

1. Membrane formation occurs in exactly half of the experiments here described. It was observed in from 1 to 8 per cent. of the maturated eggs.

2. The degree of completeness of membrane formation varies greatly in different sets of eggs and in different eggs of a given set. In some eggs the membrane is so little lifted from the surface of the egg as to be scarcely noticeable, but in others the membrane is indistinguishable from that seen in fertilized eggs.

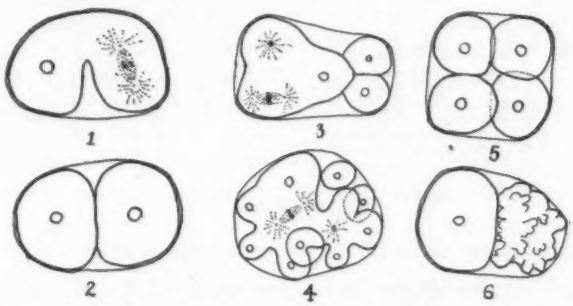
3. Eggs that form membranes, whether narrow or wide, do not further develop, but undergo cytolysis within twenty-four hours.

4. The percentage of maturated eggs that undergo parthenogenetic development varies from none to about seventy-five; in experiment II no cleavage occurred, while in experiment X 75 per cent. of all maturated eggs at least began cleavage. The aver-

age number of parthenogenetic eggs is about two per cent, of maturated eggs.

5. Eggs that undergo parthenogenetic cleavage never form "fertilization" membranes, the closely fitting vitelline membrane being the only envelope that surrounds the blastomeres.

6. Cleavage in parthenogenetic eggs never begins earlier than six and one half hours after the eggs are placed in sea water and the average time for the beginning of cleavage is about seven and a quarter hours. Cleavage begins in fertilized eggs sometimes as early as three and one half hours, and the average time of beginning is about four hours. Subtracting two hours for maturation to complete itself, we have cleavage beginning five hours after maturation in parthenogenetic eggs and two hours after maturation in fertilized eggs. There is, therefore, a retardation in development in the case of parthenogenetic eggs of three hours, and at a very critical period.



FIGS. 1–6. Cleavage stages in spontaneously parthenogenetic eggs of *Asterina*. *1*. An incompletely segmented two-cell stage in which one blastomere is in advance of the other. *2*. A normal two-cell stage. *3* and *4*. Irregular cleavage stages. *5*. A typical normal four-cell stage. *6*. A rather common type of abnormal cleavage in which one blastomere is undergoing cytolysis and the other is remaining normal.

7. Cleavage and subsequent development in parthenogenetic eggs take place much more slowly than in fertilized eggs. Even the most nearly normal parthenogenetic eggs take nearly twice as long to reach a given stage as do fertilized eggs. Development is, therefore, greatly retarded and we would naturally ex-

pect the larvæ to exhibit the various types of developmental defects that are commonly seen in inhibited individuals.

8. In none of the numerous experiments did parthenogenetic eggs give rise to even approximately normal bipennariæ. The most successful larvæ were certain double monsters that will be discussed later.

9. The average viability of parthenogenetic larvæ varies greatly in different sets. As a rule viability was lowest in those sets in which the smallest percentage of larvæ occurred and highest in those in which the largest percentage of larvæ occurred.

10. Individual viability varies greatly within a given set of eggs. Quite frequently eggs die and disintegrate during the first or subsequent cleavages, while it was not uncommon for a few larvæ in each of the best sets to live for from four to seven days.

11. Cleavage in parthenogenetic eggs is sometimes very normal in appearance, but in every set the majority of cleavage stages are irregular (Figs. 1–6). Sometimes blastomeres of the two cell stage separate, and form half-sized blastulæ, seldom going further. In other cases one or more blastomeres cease cleavage while the rest go on and form a covering of small cells about a large central cell. Numerous other cleavage anomalies occur which need not be detailed here.

Discussion.

Loeb's Observations of Spontaneous Parthenogenesis in Asterina.

Doubtless the reader recalls the work of Loeb (1905) on "Artificial membrane formation and chemical fertilization in a starfish (Asterina)." In this paper the author describes various methods employed first, for inducing membrane formation and second, for inducing cleavage and subsequent development in the same species of starfish which forms the material of the present investigation. Loeb recognizes the occurrence of spontaneous parthenogenesis in *Asterina* as is shown by the following quotations: "The eggs of the starfish show a slight tendency to develop spontaneously without any external influence." "If the eggs of *Asterina* are allowed to mature in sea water and are left to themselves, sometimes none, sometimes a fraction of a per cent., some-

times more, will segment and develop into larvæ. But the development of these eggs is much slower than that of fertilized eggs and, as a rule the larvæ are not so perfect and die sooner." "We have, therefore, two types of development in these (*Asterina*) eggs. One type is represented by the fertilized egg, and this type can be produced artificially in a number of eggs, at least, by calling forth the membrane formation by the above-named artificial means. The second type is represented by the spontaneously developing egg in which no membrane has been called forth; these latter eggs begin to segment later, and possibly develop more slowly than the other eggs, and form larvæ which are not as perfect as those belonging to the first type."

It will be seen that Loeb has touched upon some of the essential points that are brought out in my experiments. He notes that spontaneous parthenogenesis occurs in a small per cent. of eggs; that parthenogenetic cleavage takes place without membrane formation; that cleavage begins later; and that development is slower and less normal than is fertilized eggs. Loeb, however, was not primarily interested in the course or results of spontaneous parthenogenesis, but merely dealt with it incidentally as a check upon his work on artificial parthenogenesis or chemical fertilization. He, therefore, merely points out the foregoing particulars without entering into any discussion as to their significance.

Spontaneous Membrane Formation.

In only one important point is there lack of essential agreement between his results and mine: he failed to note any cases of spontaneous membrane formation which was so frequently noted in my experiments. I am at a loss to explain this discrepancy between his results and mine, both performed at Pacific Grove and both unquestionably safeguarded against accidental error. Possibly the material behaves differently at different times of the year and Loeb's work was done at quite a different time from mine, which was confined to the last few days of April and the first three weeks of May. The only difference in treatment between Loeb's cultures and mine had to do with the methods of

sterilizing the sea water in order to avoid normal fertilization. Although he does not mention the fact in these particular experiments, his practice was to use boiled or highly heated sea water; while I used sea water that had been kept in a demijohn for at least four days. It seems barely possible that heating of the water prevents spontaneous membrane formation. I would, of course, have tried this experiment, had I known of Loeb's detailed paper at the time of my experiments, but I had with me only his book on "Artificial Parthenogenesis and Fertilization" and in that book he fails to mention the occurrence of spontaneous parthenogenesis in *Asterina*. Heated sea water would doubtless be relatively poor in oxygen and this might be responsible for his failure to find spontaneous membrane formation. It is possible also that this process (membrane formation) was so belated in its appearance that it occurred after Loeb had ceased to look for it in his cultures. Unless one gets a very early start in experiments with this material the working day is likely to be over before any signs of membrane formation appear, and the next morning these eggs will have undergone cytolysis and their membranes will have disappeared. My plan was to make a before-breakfast expedition to the collecting grounds, get the material ready for work, breakfast, and be ready for experimentation by 8:00 A.M. If one begins to collect after breakfast it is likely to be nearly 11:00 A.M. before experiments with *Asterina* eggs could be commenced. If such were the case it would be 6:00 P.M. before distinct membranes would be visible, later than the time when the investigator habitually "knocks off for the day." It is barely possible then that Loeb may have missed spontaneous membrane formation in some such way as this.

Of the validity of my obervations there seems to be no doubt. In answer to the criticism that failure to heat the sea-water vitiates the observations, it may be said that if these eggs with membranes are fertilized, they should segment; but they never do. We seem to be forced to the conclusion, therefore, that membrane formation in *Asterina*, which Loeb has been at such pains to bring about by chemical means, occurs spontaneously in a considerable percentage of cases. This being true, the various manipulations

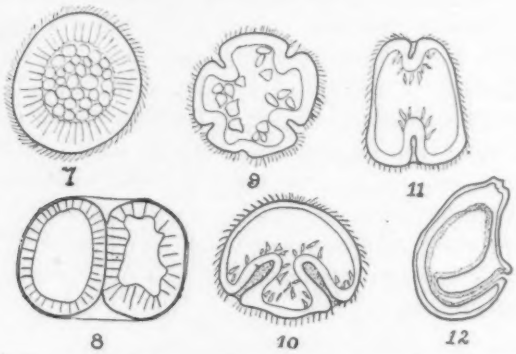
used by Loeb have merely served to hasten a natural process and to cause it to occur in a larger percentage of eggs.

Something in addition to membrane formation occurs in the eggs handled by Loeb, for they go ahead and segment as do normally fertilized eggs, while the eggs that form spontaneous membranes do not segment. From this it would appear that *the so-called "fertilization membrane" is not an essential feature of development, but merely its usual accompaniment.* Thus the voluminous literature dealing with artificial membrane formation, as though it were the most important event in the initiation of development, loses some of its force. Loeb himself recognized that development, at least in *Asterina,* could proceed without membrane formation; witness his statement, corroborated by my own observations, that spontaneous parthenogenesis proceeds without the preliminary of membrane formation. He seems to suggest, however, that this is not to be considered as typical development, since it begins later, goes more slowly and results less normally than in the case of fertilized eggs. Exactly similar results may be obtained, however, in fertilized eggs by the use of agents that retard development, such as cold, hybridization, anæsthetics, etc. So we must admit that *real development may occur without membrane formation, and that membrane formation may occur without initiation of development. The two processes are independent though they usually are associated in normal ontogeny.*

The Development Spontaneously of Parthenogenetic Eggs of Asterina.

According to Loeb, the development of the chemically fertilized eggs differs from that of the spontaneously fertilized eggs in two respects; first, in forming membranes; and second, in beginning earlier and proceeding more rapidly. With this distinction I fully agree. Evidently, in the chemically fertilized eggs, something in addition to membrane formation takes place, a something that results in prompt initiation of the changes expressed by cleavage. In the spontaneously parthenogenetic eggs, however, initiation to development is very slow in beginning, and

is less effective when it does begin. In last analysis the difference is evidently to be expressed in terms of rate of change. *The whole process of ontogeny in these eggs is from the beginning retarded, and the results are exactly similar to those which may be obtained by the use of growth-retarding agents applied to newly fertilized eggs.* The first effect of pronounced retardation of the normal growth process in the egg is the partial or complete obliteration of the characteristic axes of polarity and symmetry in the egg, a breaking down of the axial gradient. This results subsequently in loss of unity of organization, involving physiological isolation of blastomeres or of cell aggregates, in double and triple polarity and consequent double or triple monsters, and in a whole series of products of differential inhibition, such as those described by Child for sea-urchin.



FIGS. 7–12. Later developmental stages in spontaneously parthenogenetic eggs of *Asterina*. *7*. The commonly occurring solid blastula type. *8*. A pair of twin blastulæ enclosed within one vitelline membrane, evidently the result of physiological isolation of two blastomeres in the two-cell stage. *9*. A multipolar embryo gastrulating at several points. *10*. Double monster with two symmetrical archentera. *11*. Another double monster with an additional anterior archenteron. *12*. A microcephalic ciliated larva, with differentiated stomach and intestine, but no anterior parts.

Some of the types of inhibited larvæ found in cultures of spontaneously parthenogenetic *Asterina* eggs are shown and described in figures 7–12. The solid blastula is the commonest type, a type devoid of an axis of polarity (Fig. 7). Forms that are bipolar

and tripolar, etc., usually undergo gastrulation in two or more places (Figs. 9, 10, 11) and produce double and triple monsters, etc. The most nearly normal forms are decidedly abnormal early bipennaria larvæ in which the anterior parts are relatively inhibited. Such a form is shown in Fig. 12, in which the mouth never breaks through, oesophogus is not clearly differentiated, but stomach and intestine are well devevloped. Since I have in preparation a detailed paper on twinning in *Asterina*, in which I intend to discuss the physiology of twinning in general, I shall not enter further into an account of the various types of inhibited larvæ that result from spontaneous parthenogenesis, for the same types result from several other kinds of inhibiting factors. In closing I merely wish to emphasize this one point: that ***the results of spontaneous parthenogenesis are those usually found to accompany early growth retardation.*** For a summary of this paper the reader is referred to the eleven points referred to on pages 110–112 and to the italicized clauses in the general discussion.

I am greatly indebted to the Hopkins Marine Station of Leland Stanford University, and to its director, Dr. Walter K. Fisher, for the excellent facilities for research that I enjoyed while at Pacific Grove.

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AUTHOR'S ABSTRACT OF THIS PAPER ISSUED
BY THE BIBLIOGRAPHIC SERVICE, FEBRUARY 7

ON THE OCCURRENCE OF PAIRED MADREPORIC PORES AND PORE-CANALS IN THE ADVANCED BIPENNARIA LARVÆ OF ASTERINA (PATIRIA) MINIATA TOGETHER WITH A DISCUSSION OF THE SIGNIFICANCE OF SIMILAR STRUCTURES IN OTHER ECHINODERM LARVÆ.

H. H. NEWMAN.[1]

Introduction.

Ever since the theory became current that the bilaterally symmetrical larvæ of echinoderms afford phylogenetic evidence that this group of radially symmetrical animals was derived from bilaterally symmetrical ancestors, larvæ that showed more than the normal tendencies toward persistent bilaterality have had a special significance.

Normally, the first evidence of the encroachment of the adult radial symmetry upon the the larval bilaterality is seen in the development of a distinct hydrocœl with a madreporic pore and pore-canal on the left side, and none on the right. This failure of the right side to keep pace with the left has been considered as the mechanical cause for the twisting around of the serially repeated primordia of the radial water canals and the assumption of the adult radial symmetry.

The occurrence, therefore, in the larvæ of at least two classes of echinoderms, of paired right and left madreporic pores, pore-canals, and other derivatives of the hydrocœls, looks like the persistence of the ancestral bilateriality and tends to strengthen the current theory as to echinoderm phylogeny.

As early as 1892 Field, in his account of the larval development of *Asterias vulgaris*, described the transitory appearance in all

[1] From the Hopkins Marine Station of Leland Stanford University and the Hull Zoölogical Laboratory of the University of Chicago.

larvæ aged about three and a half days of a strictly bilaterally symmetrical condition of the hydrocœls, madreporic pores, and pore-canals. Soon after this period the right madreporic pore closes and the pore-canal, without an external opening, persists for a short time and then entirely disappears.

Several continental writers had observed the occasional occurrence in asteroid larvæ of paired madreporic pores, but had considered the condition as a pathological one. Field, however, maintains that there normally occurs, in *Asterias vulgaris* at least, a transitory stage in which bilateral madreporic pores exist, and considers this condition as "a true ontogenetic character, and of very considerable phylogentic significance." Gemmill (1912) was able partially to confirm Field's observations of the occurrence of paired water pores in the genus *Asterias*, finding this condition in fifty per cent. of larvæ of *Asterias glacialis* and in about ten per cent. of those *Asterias rubens*. He also states that in all cases the water-pore soon closes.

Paired echinus-organs, madreporic vesicles, and other derivatives of the right hydrocœl, were observed by MacBride (1911) in two very advanced Plutei, one of *Echinus miliaris* and the other of *Echinus esculentis*. A similar condition was observed by Grave (1911) in a single Pluteus of the sand-dollar *Mellita pentapora*, though the figure shows the two madreporic canals making exit through a single median dorsal madreporic pore.

Paired Madreporic Pores in Asterina Larvæ.

During the months of April, May and June, 1920, I had occasion to observe the development of a very large number of cultures of larvæ of *Asterina* (*Patiria*) *miniata* at Pacific Grove, California. In one culture consisting of otherwise normal, healthy larvæ three weeks old, I noticed a larva with two perfect madreporic pores and pore-canals. Further search, involving a complete census of all the larvæ in the culture, revealed twenty-six more larvæ with right madreporic canals in some cases as well developed as the left; but in others with the right pore closed and the pore-canal smaller than the one on the left. More than half of all the advanced larvæ in this culture had the double madre-

poric pores or at least double pore-canals, while the remainder of the larvæ were quite typical. After a prolonged search in other cultures of *Asterina*, I was unable to discover any other larvæ with double madreporic pores.

These double-pored larvæ were carefully watched and attempts were made to rear them by introducing diatoms collected from

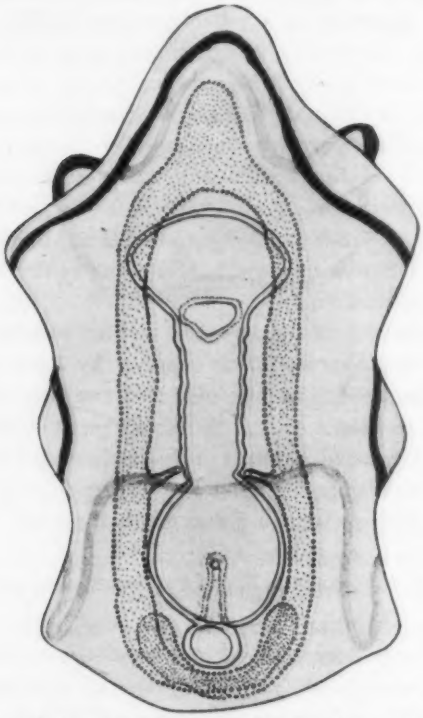

FIG. 1. Dorsal view of largest bipennaria, viewed as a transparent object. The hydroenterocoel cavities are shown stippled. Note both left and right madreporic pores, opening far apart; distinct pore-canals on both sides.

the kelp found in the tide pools inhabited by *Asterina*. They fed to some extent on several species of diatoms, but made no progress beyond the condition in which they were when the double-pored character was first noticed. Instead, they slowly retrogressed in

differentiation, became sluggish, and died without having begun to metamorphose. Throughout their lives they remained bilaterally symmetrical and observations made at the end of the fourth week, shortly before the culture began to die out, showed that the right madreporic pore was still open in several of the largest bipennariæ. While the larvæ were still active and in good health, several specimens were quieted with chloretone and mounted for

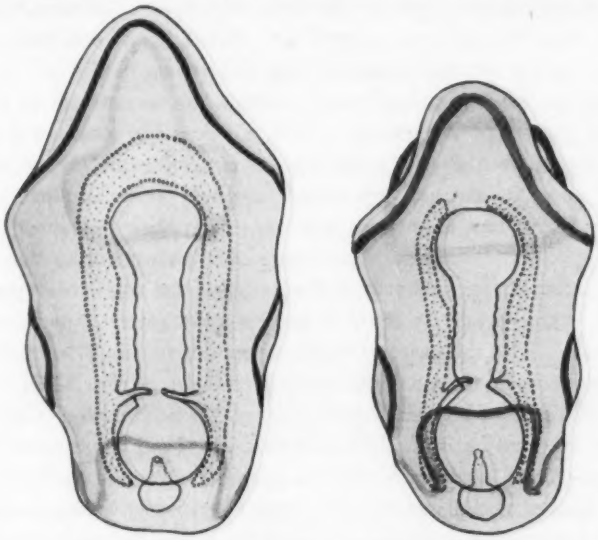

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FIG. 2. Dorsal view of a somewhat less advanced bipennaria with madreporic pores close together but both open.

FIG. 3. Dorsal view of one of the least advanced bipennariæ with right madreporic pore closed, but with distinct pore canal on the right side.

microscopic study. These were drawn with camera lucida and three of these drawings are shown in Figs. 1–3. The largest larva in the culture is shown in Fig. 1. Its length was a little over 7 mm. The anterior hydroenterocœl pouches have become completely fused in front of the oral funnel and have grown forward into a median pre-oral cœlomic cavity. The right and left body cavities are equally well developed throughout and each has grown posteriorly beyond its own side and has curled round

the intestine, so as to have its blind end pointed forward on the opposite side of the body. Thus these two posterior outgrowths cross one another and encroach on each other's territory, a condition that could not well represent any ancestral state, but one to be expected in a double monster such as I believe this to be. Each madreporic canal connects broadly with the dorsal wall of its respective hydrocœl, slopes slightly toward the median line, and opens by a distinct pore to the exterior. The two pores are not very close together in this specimen. The other two larvæ are a little smaller and less advanced than that shown in Fig. 1. That shown in Fig. 2 is about 6 mm. in length, has no pronounced forward growth of the preoral cœlom and does not show an overlapping of the posterior extensions of the right and left cœloms. The pores of the two pore-canals are very close together and might form one double madreporite. The specimen shown in Fig. 3 was of about the same stage of advancement as that in Fig. 2 but differs primarily in the fact that the right madreporic pore is closed and its canal is smaller and shorter than its left partner. Also the anterior cœloms have not fused.

In discussing these anomalous larvæ with Dr. Walter K. Fisher, director of the Hopkins Marine Station, I was interested to learn from him that he had, while collecting, noted adult specimens of *Asterina*, and of other species of asteroids, in which there were two madreporic plates and, correspondingly, two stone canals. This information immediately suggested to me the strong probability that these adults with paired madreporic plates must have arisen from larvæ with double madreporic pores such as I had under observation. The question would then arise as to whether this double condition in the adults would bear the same phylogenetic interpretation as has been offered for the double condition in the larvæ. If not; why not? But this involves us in a discussion of the significance of these anomalous paired structures in echinoderms.

Discussion.

Three distinct interpretations have been offered for the appearance of these anomalous right-hand elements that normally appear only on the left.

1. The first interpretation is well phrased by MacBride[1] as follows:

"The appearance of a right and left madreporic pore is the first indication of what is really the key to the understanding of Echinoderm development, viz., the fact that the two sides of the larvæ originally gave rise to precisely similar organs, but that some of these organs grew and developed on the left side while they atrophied on the right, and that thus an asymmetry was produced."

This seems to be the natural interpretation of the facts, but there are certain other facts that this interpretation does not cover. In a larva of the sand-dollar *Mellita pentapora,* Grave (1919) found that not only were the mesodermal structures, including hydrocoel, pore-canals, etc., bilaterally repeated, but also paired ectodermal pouches occurred which had no reference to the water-vascular system, but were the primordia of the nervous system. Grave is inclined to doubt the validity of interpreting this extra ectodermal pouch as a reversion to an ancestral condition, and I would fully agree with him. The occurence of adult starfishes with paired madreporic plates and stone canals also weakens the phylogentic interpretation of double pores in larvæ; for they are evidently strictly homologous structures. If the double-plate condition in the adult is to be interpreted as a reversion, of what is it an ancestral reminscence? Surely not of an ancestral starfish with radial symmetry of other organs, but bilateral madreporic structures!

2. The second interpretation of paired madreporic structures involves the idea that they are homœotic variations, in Bateson's sense. Such variations may be viewed according to Grave "as cases of perfected symmetry, the result of a long continued strain due to imperfect balance, either morphological or physiological or both, between the organism and its environment." I confess that I am unable to see the force of this interpretation. It is highly mystical in tone and savors of some internal perfecting principle or "entelechy." If such a principle were operative, the real problem would be to account for the failure of echinoderm

[1] "Text-book of Embryology," Vol. I., p. 466.

larvæ in general to continue to maintain their original bilaterality. That is a different problem altogether.

3. A third theory involves the idea that these duplicated structures are the products of twinning and this is what I believe they are. In laboratory cultures of *Asterina* (and in at least two other species of asteroids with which I have worked) there are numerous instances of twins and double monsters. The exact cause of twinning and doubling is not fully known and will not be discussed here, as I have in preparation a more extensive paper on this subject. It may be said, however, that a long series of types has been found in which the original right and left primordia of the future embryo become more or less completely physiologically isolated, and in proportion to the duration or completeness of the isolation, each half develops more or less independently of the other. The result is a series of more or less completely doubled larvæ, as follows: twin blastulæ which soon separate and develop into half-sized larvæ; gastrulæ with paired archentera; gastrulæ with paired blastopores, but with archentera fused anteriorly; early bipennariæ with the anterior end of the archenteron branched and with the beginning of a double set of hydroenterocœl pouches; and finally advanced larvæ with double madreporic pores and water canals. I have placed the type of anomalous larvæ under discussion at the end of what appears to me to be a logical series, representing the results of varying degrees of physiological isolation of bilateral halves of embryos. The type of result attained seems to depend on the time of incidence of the cause or causes of the physiological isolation in question and upon the degree of severity or duration of the causal agent, whatever it may be.

There is therefore no more justification for the use of "double-pored" larvæ of echinoderms as evidence of an ancestral condition than there is for giving a similar significance to instances of dicephaly or spina bifida in vertebrates. For they are all, in my opinion, phases of "twinning" in the broad sense and will doubtless be explicable on some general physiological basis that we shall hope to discuss in the future. Let me close with a word of caution. Beware of giving a phylogenetic interpretation to an

anomaly found in laboratory-bred larvæ of any sort. Such aberrations are more than likely to be the result of subnormal conditions and nothing more.

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